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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
GOLDBERG, JEANINE ANNE	
ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 07/28/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/085,774	SYLVAN, ANNA	
	Examiner	Art Unit	
	Jeanine A Goldberg	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *24 April 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-23* is/are pending in the application.

4a) Of the above claim(s) *16* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-15 and 17-23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *27 February 2002* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *6/11/02* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

1. This action is in response to the papers filed April 24, 2003. Currently, claims 1-23 are pending. Claim 16 has been withdrawn as drawn to non-elected subject matter.

Election/Restrictions

2. Applicant's election of Group I in the paper filed April 24, 2003 is acknowledged. The response traverses the restriction requirement because the "subject matter of Claim 16 is related to the subject matter of the claims of Group I, and thus would not require an undue burden." This argument has been thoroughly reviewed, but is not found persuasive because the products may be used in a materially different method. The products and methods have acquired a separate status in the art as recognized by their different classification, as recognized by their divergent subject matter and because a search of the subject matter of invention I is not co-extensive with a search of inventions II. The requirement is still deemed proper and is therefore made FINAL.
This application contains claim 16 drawn to an invention nonelected with traverse. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

Priority

3. This application claims priority to provisional application 60/271,703.

As provided in MPEP 201.11, "When the nonprovisional application is entitled to an earlier U.S. effective filing date of one or more provisional applications under 35

U.S.C. 119(e), a statement such as "This application claims the benefit of U.S. Provisional Application No. 60/---, filed ---, and U.S. Provisional Application No. 60/ ---, filed ---." should appear as the first sentence of the description or in an application data sheet." The instant application does not provide a filing date for the provisional application in the first line of the specification. Appropriate correction is requested.

Drawings

4. The drawings are acceptable.

Sequence Rules

5. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825.

The instant application contains primer sequences on page 44-45, for example which fails to identify the sequences by SEQ ID NO:. Appropriate correction is required.

Specification

6. Applicant is reminded of the proper language and format for an abstract of the disclosure.

The abstract should be in narrative form and generally limited to a single paragraph on a separate sheet within the range of 50 to 150 words. It is important that

the abstract not exceed 150 words in length since the space provided for the abstract on the computer tape used by the printer is limited. The form and legal phraseology often used in patent claims, such as "means" and "said," should be avoided. The abstract should describe the disclosure sufficiently to assist readers in deciding whether there is a need for consulting the full patent text for details.

The language should be clear and concise and should not repeat information given in the title. It should avoid using phrases which can be implied, such as, "The disclosure concerns," "The disclosure defined by this invention," "The disclosure describes," etc.

Claim Objections

7. Claim 13 is objected to because of the following informalities. The claim appears to be missing a word between “will not” and “preferentially amplified” in line 3 of Claim 13. The word “be” could be inserted to provide clarity to the claim. Appropriate correction is required.

Claim Rejections - 35 USC § 112- Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

8. Claims 1-15, 17-23 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A) Claims 1-15 are indefinite because it is unclear as to whether the claims are intended to be limited to methods of determining the frequency of an allele in a population or obtaining a pattern of nucleotide incorporation. The claims are drawn to a method for determining the frequency of an allele in a population. However, the final step is obtaining a pattern of nucleotide incorporation. Accordingly, it is unclear as to whether the method is a method of determining the frequency of an allele or a method of obtaining a pattern of nucleotide incorporation.

B) Claims 2-6, 18-23 recite the limitation "the reaction mixture" in line 3 of Claim 2 and line 3 of Claim 18. These claims lacks proper antecedent basis because the claims fail to set forth any reaction mixture. Thus, there is insufficient antecedent basis for this limitation in the claims.

C) Claims 11-15 recite the limitation "said calibration" in line 2 of Claim 11. This claim lacks proper antecedent basis because the claim fails to set forth a calibration of a particular polymorphism. Thus, there is insufficient antecedent basis for this limitation in the claim. Moreover, the claim includes "(or marker)" following a reference because it is unclear what limitation this recitation imparts to the claim. It is unclear whether the polymorphism may be a marker or whether the reference is further defined as a marker. Additionally, the claim recites "said primer extension reaction." However, both claims 9 and 1 recite a primer extension reaction. Therefore, it is unclear which primer extension reaction is being referred to. Thus, the metes and bounds of the claimed invention are unclear.

D) Claims 14-15 are indefinite because it is unclear from the claim how the claim relates back to each of Claims 1 and 11. Claim 1 is directed to detecting frequencies. Claim 11 requires calibration steps which ultimately relates back to Claim 1. Claim 14 requires obtaining a reference sequence and selecting as the main reference homozygotes. It is unclear whether Claim 14 is intended to be a method step further defining the method of Claim 11 or whether the steps of Claim 14 are intended to be an additional step within the method. Claim 14 appears to be directed to a means of obtaining a calibration curve using known amounts of markers to define the curves. The language of Claim 15 makes the method steps of Claim 14 less clear. Claim 15 requires the sample molecule to be tested is pooled individually with the reference samples. This requirement does not appear to make sense given the requirement in claim 1 that pooling of a population is required. The claim language is unclear as to how all of the methods steps relate to one another. Thus, the metes and bounds of the claimed invention are unclear.

E) Claims 17-23 are indefinite because in the event that the claim is interpreted to mean determining which nucleotides are incorporated in the reaction, and analyzing the nucleotide incorporation information obtained in order to determine the amount of occurrence of said allele sample, the claim appears to be missing essential steps. It is unclear based upon the determination of which nucleotides are incorporated, how the amount of the allele would be determined without additional steps. The claims are also directed to "said reaction" in lines 8 of Claim 17, however it is unclear which reaction is being referred to because there is no previous reaction. The claim refers to primer

extension reactions, but no particular reaction. It is unclear whether the reaction is the primer extension reactions, the binding of the primer to the predetermined site or whether the reaction is directed to the incorporation of nucleotides. In the event that the claim is directed to the "primer extension reactions" the claim may be amended to recite said primer extension reactions. Moreover, the claim is directed to "said nucleotide incorporation information." This claim language appears to lack proper antecedent basis because the claim fails to set forth any nucleotide incorporation information. Finally, the claim refers to the information "thus obtained in order to determine the amount of occurrence of said allele in said sample." However, it is unclear whether the claim requires that the final step of the method is directed to determining the amount of an allele or whether the claim merely requires analyzing the information. Accordingly, because the method is for determining the amount of an allele in a sample, but the final step in the method is directed to analyzing information, it is unclear whether the method requires determining the amount of an allele or analyzing information. Thus the metes and bounds of the claimed invention are unclear.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

9. Claims 1-3, 7, 17-19 are rejected under 35 U.S.C. 102(e) as being anticipated by Huang et al (US Pat. 6,287,778, September 11, 2001, filed October 19, 1999).

Huang et al. (herein referred to as Huang) teaches a method for determining the genotype of one or more individuals (a population) at a polymorphic locus which employs amplification of a region of DNA, labeling of allele-specific extension primers containing tags, and hybridization of the products to an array of probes. The genotype is identified from the pattern of hybridization and can be used to determine the frequency of different alleles in a population (abstract). As seen in Figure 1, allele specific PCR (a primer extension reaction which sequentially adds nucleotides to the reaction mixture) is performed and generates a primer extension product only where the primer is perfectly matched (limitations of Claims 1-2, 17-18). The 3' labeled primer extension product is detected using an array, thereby obtaining a pattern of nucleotide incorporation (limitations of Claim 1). Huang teaches that "if the nucleic acid sample being tested is derived from a population or group of individual organisms, an allele frequency or the ratio of allelic forms in the population may be quantified." (col. 4, lines 17-23). The quantities of the label at known locations on the solid support can be compared, and the allele frequency can be determined for a population from whom the DNA in the sample was obtained (col. 5, lines 50-53)(limitations of Claim 3, 19). Huang

teaches a generic solid support, corresponding to the pre-selected tag sequences can be fabricated and used to detect the presence, absence, or ratio of specific allelic forms in a test sample (col. 4, lines 55-62)(limitations of Claim 7). Huang teaches "pooling for the hybridization step is desirable so that thousands of hybridizations can be performed simultaneously." (col. 5, lines 59-63). Therefore, since Huang teaches every limitation of the claimed invention, Huang anticipates the instant claims.

10. Claims 1-5, 7, 17-21 are rejected under 35 U.S.C. 102(b) as being anticipated by Nyren et al. (Analytical Biochemistry, Vol. 244, pages 367-373, 1997).

The courts have stated that claims must be given their broadest reasonable interpretation consistent with the specification *In re Morris*, 127 F.3d 1048, 1054-55, 44 USPQ2d 1023, 1027-28 (Fed. Cir. 1997); *In re Prater*, 415 F.2d 1393, 1404-05, 162 USPQ 541, 550-551 (CCPA 1969); and *In re Zletz*, 893 F.2d 319, 321-22, 13 USPQ2d 1320, 1322 (Fed. Cir. 1989) (see MPEP 2111). The instant claims are given the broadest reasonable interpretation consistent with the indefinite claim language and the specification wherein a population is defined to encompass a collection of cells from one or more entities (page 9, lines 10-15 of instant specification).

Nyren et al. (herein referred to as Nyren) teaches a method of detecting a single-base change using an enzymatic luminometric inorganic pyrophosphate (Ppi) detection assay (ELIDA). Nyren teaches that "for a mixed sample (both mutated and nonmutated DNA sequences present) the exact amount of the different alleles can be easily determined by comparing the extension rates from two different primers with each

other." (page 371, col. 2). Nyren explains that "the high sensitivity of detection allows the identification of mutations present in only a small fraction of the analyzed cells." (page 372, col. 2). Nyren teaches that the assay is suitable for large-scale applications where many samples are screened for known nucleotide variations (page 372, col. 2). Therefore, since Nyren specifically teaches that the instant method is a sensitive method for detection of mutations present in a fraction of the analyzed cells, based upon the definition provided in the specification, a population of cells has been analyzed and pooled as required by the instant claims.

Nyren teaches that primer extension efficiency by a DNA polymerase of a matched over a mismatched 3' terminal is detectable. Nyren teaches that the Ppi formed in the polymerization reaction is converted to ATP by ATP sulfurylase (page 367, col. 2). Nyren teaches using two detection primers differing with one base at the 3' end are designed (i.e. primers which bind to a predetermined site located in the nucleic acid molecules), hybridized with the template, incubated with DNA polymerase and deoxynucleotides and the primer extension rates are measured with ELIDA (i.e. a pattern of nucleotide incorporation)(limitations of Claims 1-5, 17-21). Immobilized single-stranded DNA fragments are used as template (limitations of Claim 7)(abstract, Figure 1). Nyren teaches that if the detection primer exactly matches to the template, a high extension rate will be observed. However, if the 3' end of the detection primer does not exactly match to the template, the primer extension rate will be much lower (page 369, col. 1). Nyren teaches that "the difference in primer extension efficiency by the DNA polymerase of a matched over a mismatched 3' terminal can then be used for

single-base discrimination." (page 369, col. 1). Nyren teaches the method of detecting Ppi is a fast real-time method for analysis of single-base changes. Therefore, since Nyren teaches all of the limitations of the claims, Nyren anticipates the claimed invention.

11. Claims 1-3, 7-8, 17-19 are rejected under 35 U.S.C. 102(b) as being anticipated by Shaw et al (Genome Research, Vol. 8, pages 111-123, 1998).

Shaw et al. (herein referred to as Shaw) teaches a method of determining the frequency of alleles in a pooled DNA sample by pooling nucleic acid molecules from more than one individual, performing primer extension reactions using primers which hybridize to the nucleic acid and obtaining a pattern of nucleotide incorporation (limitations of Claim 1, 17). As provided in the methods section, Shaw obtains DNA samples from 76 unrelated parents. Shaw teaches that the concentration of each DNA sample was read on a spectrophotometer and a fluorometer (page 121, col. 1). Each sample was diluted to a concentration of 10 µg/ml and reread to confirm the DNA concentration (page 121, col. 1)(limitations of Claim 8). Equal amounts of DNA from each sample constituting a pool were manually combined prior to PCR amplification (page 121, col. 1)(limitations of Claim 2, 18). Shaw teaches that each sample was amplified with 50 ng of pooled DNA and visualized on an acrylamide gel (limitations of Claim 3, 7, 19). The electrophoresis gel was analyzed to estimate the peak height for each allele. The allele frequencies were estimated from the relative proportion of the peak height for each allele versus the sum of peak heights for all alleles (page 121, col.

2). Shaw used a 5' fluorescently labeled forward PCR primer and relative peak heights to quantify allele amplification at polymorphic microsatellite markers and estimate allele frequencies in pooled DNA samples. Shaw provides extensive analysis comparing the accuracy of alleles estimates from DNA pooling with actual frequencies. Shaw teaches that pooling promises to drastically reduce the labor and cost of genotyping in the initial identification of disease loci. Therefore, since Shaw teaches every limitation of the instant claims, Shaw anticipates the claimed invention.

12. Claims 1-3, 8-9, 17-19 are rejected under 35 U.S.C. 102(b) as being anticipated by Germer et al. (Genome Research, Vol. 10, No. 2, pages 258-266, February 23, 2000).

Germer et al. (herein referred to as Germer) teaches a method for determining the allele frequency of biallelic polymorphisms in pooled samples. Specifically, Germer teaches a mixture of DNAs pooled from individual sample were subjected to primer pairs (e.g. a primer pair specific to one or the other SNP allelic variant), and detecting the frequency (page 259, col. 1)(limitations of Claim 1, 17). Germer teaches enhancing specificity of the kinetic PCR reaction by using Stoffel fragment Taq DNA polymerase (page 259, col. 1)(limitations of Claim 2-3, 18-19). Germer teaches the amplification efficiencies for the two allele-specific PCRs may differ slightly but this can be measured and compensated for by performing the assay on a DNA known to be heterozygous for the SNP of interest. Germer teaches that the deltaC for this DNA should equal zero if the PCRs are equally efficient (page 259, col. 2). Germer teaches that any deviation

can then be subtracted from all deltaC measurements to compensate for differential amplification efficiencies (page 259, col. 2). Germer teaches that error introduced by unequal amplification efficiency of the two allele-specific primers for each polymorphism may be corrected for (page 261, col. 1). Germer teaches method for avoiding the formation and potential interference of template independent generation of primer artifact by using UNG and heat-activated polymerase enzyme. Moreover, using Stoffel fragment of Taq polymerase minimizes the problem because it is highly discriminatory and not very processive (page 260, col. 2). The relative amounts of each allele in a sample are quantified (abstract). As seen in Table 2, the allele frequency measurements on a pool of 100 human DNAs in three genes illustrate very highly correlated results. Germer teaches "in conducting association studies using pools of DNA, accurate quantitation of the individual DNAs is important lest artifactual allele discrepancies between pools arise (page 263, col. 2). Germer teaches that the "simplest safeguard against errors arising from the pooling process would be to validate the pools by doing genotyping of the individual sample and showing concordance between allele counting and frequency measurement on the pool. Germer teaches that Tm-shift genotyping is a good choice because it uses the same allele-specific PCR conditions and two of the same three primers as the described method (page 263, col. 2)(limitations of Claim 9). The methods section teaches that the samples were constructed by mixing two homozygous human DNA samples in various proportions by combining known amounts of homozygous DNA samples (limitations of Claim 8). Germer teaches his method of determining SNP allele frequencies in pooled sample

has a number of advantages (1) it is not based on expensive fluorescently labeled primers or probes (2) it is a homogenous assay that requires no post-PCR processing (3) it operates under uniform conditions without the need for marker specific assay optimization (4) it is accurate and (5) it promises to be inexpensive, time-saving and precise to allow detection of relatively weak genetic associations (Page 258-259).

13. Claims 1-6, 8, 17-22 are rejected under 35 U.S.C. 102(a) as being anticipated by Bellman et al. (Poster presented April 2000 at Human Genome Project Meeting).

Bellman provides a poster entitled reliable SNP assessment and allele frequency determination by Pyrosequencing. Bellman teaches that pyrosequencing is a real-time sequencing method employing an enzyme cascade system to monitor the release of inorganic pyrophosphate (Ppi) during nucleotide incorporation. As seen in Figure 1, the pyrosequencing cascade employs apyrase, primers, and deoxynucleotides to generate an assayable signal (limitations of Claims 4-6, 20-22). Bellman teaches using Pyrosequencing is well suited to determine allele frequencies. Bellman teaches pooling 11 known individual in equal amounts and run as one sample (limitations of Claims 1-3, 8, 17-19). As seen in Figure 4, the linearity of the system for detection of alleles is illustrated. Bellman teaches that the SNP outcome after analyzing the mixture of the individuals matched perfectly with the expected frequency of each allele, thereby quantitatively determining the amount of nucleotide incorporated. Therefore, the method taught by Bellman is directed to a method of pooling nucleic acid molecules,

performing a primer extension reaction and obtaining a pattern of nucleotide incorporation.

14. Claims 1-3, 8, 17-19 are rejected under 35 U.S.C. 102(a) as being anticipated by Breen et al. (BioTechniques, Vol. 28, No. 3, pages 464-470, March 2000).

Breen et al. (herein referred to as Breen) teaches a method for determining the frequency of an allele in a population of nucleic acids by pooling nucleic acid molecules, performing a primer extension reaction and obtaining a pattern of nucleotide incorporation. Specifically, Breen teaches methods of pool construction and methods of testing the sensitivity of the DNA pooling method. Breen teaches concentrations of samples may be estimated by fluorimetry. Moreover, Breen teaches testing the accuracy of the pooling protocol by comparing the frequencies derived from individual genotyping with tests using spiking where additional alleles were introduced into DNA pools (page 464, col. 3). Breen teaches dividing the pools into aliquots and the amount of DNA from a homozygote that was equivalent to one, two, five and ten alleles was added to the aliquots. PCR (primer-extension reaction) was carried out on the pools (limitations of Claims 1-3, 8, 17-19). Breen teaches analyzing SNP in the DRD2 gene and the COLIA1 gene and estimating the frequencies (page 464, col. 3). Breen teaches that the results of the Taqman assay to determine genotypes was highly consistent and reproducible (page 469, col. 2). The Taqman assay uses both primers and probes (primer extension reaction)(limitations of Claim 1). Breen teaches using allele specific fluorescent probes to determining the number of copies of the two alleles and thus the

frequencies of the two alleles. Therefore, since Breen teaches every limitation of the instant claims, Breen anticipates the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

15. Claims 6, 22-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nyren et al. (Analytical Biochemistry, Vol. 244, pages 367-373, 1997) in view of Nyren-2 (WO 98/28440, published July 2, 1998)

The courts have stated that claims must be given their broadest reasonable interpretation consistent with the specification *In re Morris*, 127 F.3d 1048, 1054-55, 44 USPQ2d 1023, 1027-28 (Fed. Cir. 1997); *In re Prater*, 415 F.2d 1393, 1404-05, 162 USPQ 541, 550-551 (CCPA 1969); and *In re Zletz*, 893 F.2d 319, 321-22, 13 USPQ2d 1320, 1322 (Fed. Cir. 1989) (see MPEP 2111). The instant claims are given the broadest reasonable interpretation consistent with the indefinite claim language and the specification wherein a population is defined to encompass a collection of cells from one or more entities (page 9, lines 10-15 of instant specification).

Nyren et al. (herein referred to as Nyren) teaches a method of detecting a single-base change using an enzymatic luminometric inorganic pyrophosphate (Ppi) detection assay (ELIDA). Nyren teaches that "for a mixed sample (both mutated and

nonmutated DNA sequences present) the exact amount of the different alleles can be easily determined by comparing the extension rates from two different primers with each other." (page 371, col. 2). Nyren explains that "the high sensitivity of detection allows the identification of mutations present in only a small fraction of the analyzed cells." (page 372, col. 2). Nyren teaches that the assay is suitable for large-scale applications where many samples are screened for known nucleotide variations (page 372, col. 2). Therefore, since Nyren specifically teaches that the instant method is a sensitive method for detection of mutations present in a fraction of the analyzed cells, based upon the definition provided in the specification, a population of cells has been analyzed and pooled as required by the instant claims.

Nyren teaches that primer extension efficiency by a DNA polymerase of a matched over a mismatched 3' terminal is detectable. Nyren teaches that the Ppi formed in the polymerization reaction is converted to ATP by ATP sulfurylase (page 367, col. 2). Nyren teaches using two detection primers differing with one base at the 3' end are designed (i.e. primers which bind to a predetermined site located in the nucleic acid molecules), hybridized with the template, incubated with DNA polymerase and deoxynucleotides and the primer extension rates are measured with ELIDA (i.e. a pattern of nucleotide incorporation).

Nyren teaches that if the detection primer exactly matches to the template, a high extension rate will be observed. However, if the 3' end of the detection primer does not exactly match to the template, the primer extension rate will be much lower (page 369, col. 1). Nyren teaches that "the difference in primer extension efficiency by the DNA

polymerase of a matched over a mismatched 3' terminal can then be used for single-base discrimination." (page 369, col. 1). Nyren teaches the method of detecting Ppi is a fast real-time method for analysis of single-base changes.

Nyren does not specifically teach adding a nucleotide-degrading enzyme to the assay for detecting the release of Ppi.

However, Nyren-2 teaches a method of sequencing DNA based on the detection of the release of pyrophosphate. Nyren-2 teaches identifying a base at a target position in a sample DNA sequence wherein an extension primer, which hybridizes to the sample DNA immediately adjacent to the target position is provided and the sample DNA and extension primer are subjected to polymerase reaction in the presence of a deoxynucleotide where the deoxynucleotide will only become incorporated and release pyrophosphate (PPI) if it is complementary to the base in the target position. Any release of Ppi being detectable enzymically. Nyren-2 teaches including a nucleotide degrading enzyme during the polymerase reaction step, such that unincorporated nucleotides are degraded (page 3, para 2). Nyren-2 specifically teaches that apyrase is a nucleotide-degrading enzyme (page 4). Nyren-2 teaches that including a nucleotide-degrading enzyme allows the sequencing procedure to proceed without washing the template between successive nucleotide additions. Additionally, since washing steps are avoided, it is not necessary to add new enzymes (page 5).

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the primer extension assay of Nyren with the teachings of Nyren-2 to include adding a nucleotide-degrading enzyme during the

primer extension reaction. Nyren-2 specifically teaches adding a nucleotide-degrading enzyme allows the sequencing procedure to proceed without washing the template between successive nucleotide additions. Thus, the ordinary artisan would have been motivated to eliminate the need for continuous wash steps between nucleotide additions. Elimination a wash steps would facilitate a less labor intensive method and require less time to complete the method. Therefore, adding a nucleotide-degrading enzyme to the known method of Nyren as taught in the method of Nyren-2 would

16. Claims 9-11, 14-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Breen et al. (BioTechniques, Vol. 28, No. 3, pages 464-470, March 2000).

Breen et al. (herein referred to as Breen) teaches methods of pool construction and methods of testing the sensitivity of the DNA pooling method. Breen teaches concentrations of samples may be estimated by fluorimetry. Moreover, Breen teaches testing the accuracy of the pooling protocol by comparing the frequencies derived from individual genotyping with tests using spiking where additional alleles were introduced into DNA pools (page 464, col. 3). Breen teaches dividing the pools into aliquots and the amount of DNA from a homozygote that was equivalent to one, two, five and ten alleles was added to the aliquots. PCR (primer-extension reaction) was carried out on the pools. Moreover, additional calibration pools were constructed and DNA from different homozygotes was mixed together in different ratios (0:100, 20:80, 40:60, etc)(page 464, col. 3). Breen teaches analyzing SNP in the DRD2 gene and the COLIA1 gene (page 464, col. 3). Figure 2 illustrates a calibration curve to correct for

the distorted allele frequencies derived from pooling (page 466). Breen teaches that the results of the Taqman assay to determine genotypes was highly consistent and reproducible (page 469, col. 2). The Taqman assay uses both primers and probes (primer extension reaction). Breen teaches using allele specific fluorescent probes to determining the number of copies of the two alleles. Breen illustrates the difference between the estimate and the results of the differing levels of probes was significant, with a p value equivalent to $P<0.0001$ (page 469, col. 3). Thus, Breen teaches that copies of the two alleles may be determined using fluorescent probes, i.e. the total of the two alleles provides an accurate indication of the concentration of the original sample.

While Breen specifically teaches using the same concentration of each DNA sample, Breen does not specifically teach determining the concentration of the nucleic acid by a primer extension reaction prior to pooling.

However, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the method of Breen which detects concentrations of each DNA sample using a fluorimeter with a method using allele-specific fluorescent probes as taught by Breen. Breen teaches "it seems the only limiting factor on accuracy in this system is the variation introduced when the pools are constructed and the DNA concentration measurements made." (page 470, col. 1). To obtain accurate information regarding the true frequencies of a pooled population, it is essential that each member of the population is represented in equal concentrations within the sample. For example if the population is comprised of two homozygote

individuals, aa and AA, and the pool is designed to contain 10 µg of DNA from the individual with "aa" and 90 µg of DNA from the individual with "AA", the estimated frequency of the alleles would be 0.1 for "a" and 0.9 for "A". This would not accurately reflect the true population frequency. In contrast, a pool designed to contain 50 µg of DNA from the individual with "aa" and 50 µg of DNA from the individual with "AA", the estimated frequency of the alleles would be 0.5 for "a" and 0.5 for "A", a true estimate of the frequencies of the two alleles in the population. Therefore, in order to obtain a true estimate of the frequency of alleles in a population, adjusting the amount or concentration of a nucleic acid in a sample is essential. It would have been obvious to the ordinary artisan to dilute or increase nucleic acid concentrations to ensure equal concentrations within each of the samples prior to pooling. Thus, because Breen illustrates the accuracy of the allele-specific fluorescent probe system in determining the copy numbers of various alleles in a particular sample, the concentration (the total of all possible alleles in the sample) may be accurately determined. Since the ordinary artisan performing the method of Breen for determining the allele frequencies in a pooled DNA sample using a 5' fluorescently labeled primer teaches the necessity of having equal concentrations of each sample, the ordinary artisan would recognize that a method of determining concentrations of samples using a Taqman assay would be an alternative means of obtaining information regarding concentration of a sample. Breen teaches that the results of the Taqman assay to determine genotypes was highly consistent and reproducible (page 469, col. 2).

With respect to Claim 14-15, Breen teaches DNA from different homozygotes was mixed together in different ratios (as seen in Figure 2). Figure 2 illustrates pooling of samples at different concentrations to determine the relative concentration in each reference sample, thereby generating a calibration cure to correct for the distorted allele frequencies derived from pooling analysis.

Therefore, using the Taqman assay would be an equivalent means of determining the concentration of a particular sample and the ordinary artisan would have been motivated to have used the assay for the benefit of using a highly consistent and reproducible assay for determining the concentration of nucleic acids present in a sample.

17. Claims 10-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Germer et al. (Genome Research, Vol. 10, No. 2, pages 258-266, February 23, 2000).

Germer et al. (herein referred to as Germer) teaches a method for determining the allele frequency of biallelic polymorphisms in pooled samples. Specifically, Germer teaches a mixture of DNAs pooled from individual sample were subjected to primer pairs (e.g. a primer pair specific to one or the other SNP allelic variant), and detecting the frequency (page 259, col. 1)(limitations of Claim 1). Germer teaches enhancing specificity of the kinetic PCR reaction by using Stoffel fragment Taq DNA polymerase (page 259, col. 1)(limitations of Claim 2-3). Germer teaches the amplification efficiencies for the two allele-specific PCRs may differ slightly but this can be measured and compensated for by performing the assay on a DNA known to be heterozygous for

the SNP of interest. Germer teaches that the deltaC for this DNA should equal zero if the PCRs are equally efficient (page 259, col. 2). Germer teaches that any deviation can then be subtracted from all deltaC measurements to compensate for differential amplification efficiencies (page 259, col. 2). Germer teaches that error introduced by unequal amplification efficiency of the two allele-specific primers for each polymorphism may be corrected for (page 261, col. 1). Germer teaches method for avoiding the formation and potential interference of template independent generation of primer artifact by using UNG and heat-activated polymerase enzyme. Moreover, using Stoffel fragment of Taq polymerase minimizes the problem because it is highly discriminatory and not very processive (page 260, col. 2). The relative amounts of each allele in a sample are quantified (abstract). As seen in Table 2, the allele frequency measurements on a pool of 100 human DNAs in three genes illustrate very highly correlated results. Germer teaches "in conducting association studies using pools of DNA, accurate quantitation of the individual DNAs is important lest artifactual allele discrepancies between pools arise (page 263, col. 2). Germer teaches that the "simplest safeguard against errors arising from the pooling process would be to validate the pools by doing genotyping of the individual sample and showing concordance between allele counting and frequency measurement on the pool. Germer teaches that Tm-shift genotyping is a good choice because it uses the same allele-specific PCR conditions and two of the same three primers as the described method (page 263, col. 2). The methods section teaches that the samples were constructed by mixing two homozygous human DNA samples in various proportions by combining known amounts

of homozygous DNA samples (limitations of Claim 8). Germer teaches his method of determining SNP allele frequencies in pooled sample has a number of advantages (1) it is not based on expensive fluorescently labeled primers or probes (2) it is a homogenous assay that requires no post-PCR processing (3) it operates under uniform conditions without the need for marker specific assay optimization (4) it is accurate and (5) it promises to be inexpensive, time-saving and precise to allow detection of relatively weak genetic associations (Page 258-259).

While Germer specifically teaches using the same concentration of each DNA sample is important lest artifactual allele discrepancies between pools arise, Germer does not specifically adjusting the amount of nucleic acids to contain substantially the same amount.

However, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the method of Germer which detects concentrations of each DNA sample using a Tm-shift genotyping assay to detect quantities with a further method step of adjusting the concentration. Since Germer teaches "in conducting association studies using pools of DNA, accurate quantitation of the individual DNAs is important lest artifactual allele discrepancies between pools arise (page 263, col. 2) and the "simplest safeguard against errors arising from the pooling process would be to validate the pools by doing genotyping of the individual sample and showing concordance between allele counting and frequency measurement on the pool", it would have been obvious to adjust the amount or concentration of the nucleic acids present in the event that a discrepancy was ascertained. To obtain accurate

information regarding the true frequencies of a pooled population, it is essential that each member of the population is represented in equal concentrations within the sample. For example if the population is comprised of two homozygote individuals, aa and AA, and the pool is designed to contain 10 µg of DNA from the individual with "aa" and 90 µg of DNA from the individual with "AA", the estimated frequency of the alleles would be 0.1 for "a" and 0.9 for "A". This would not accurately reflect the true population frequency. In contrast, a pool designed to contain 50 µg of DNA from the individual with "aa" and 50 µg of DNA from the individual with "AA", the estimated frequency of the alleles would be 0.5 for "a" and 0.5 for "A", a true estimate of the frequencies of the two alleles in the population. Therefore, in order to obtain a true estimate of the frequency of alleles in a population, adjusting the amount or concentration of a nucleic acid in a sample is essential. It would have been obvious to the ordinary artisan to dilute or increase nucleic acid concentrations to ensure equal concentrations within each of the samples prior to pooling.

With respect to Claim 11, Germer teaches assaying for three SNPs in the samples. Therefore, any one of these polymorphisms may be considered a reference polymorphism.

With respect to Claim 12, Germer teaches methods of ensuring that the error introduced by unequal amplification efficiency of the two primers for each polymorphism is corrected. Germer teaches correcting for the error between unequal amplification efficiency. Therefore, it would have been obvious to the ordinary artisan to correct for

the polymorphism with respect to background signals and unequal amplification efficiencies.

With respect to Claim 13, the polymorphisms in the PON, B71 and CSTS genes are not located within a homopoymeric sequence, as exemplified by the sequence of the primers provided. Therefore, Germer teaches using a polymorphism no present in a homopolymeric sequence.

With respect to Claim 14-15, Germer teaches DNA from different homozygotes was mixed together in different ratios and analyzed (page 264, col. 2). Table 1 illustrates pooling of samples at different concentrations to determine the relative concentration in each reference sample, thereby generating a calibration cure to correct for the distorted allele frequencies derived from pooling analysis.

Conclusion

18. No claims allowable over the art.

19. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Goldberg whose telephone number is (703) 306-5817. The examiner can normally be reached Monday-Friday from 8:00 a.m. to 5:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax number for this Group is (703) 305- 3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

J. Goldberg

Jeanine Goldberg
Patent Examiner
July 16, 2003